REMARKS

1. Status of the Claims

Claims 1, 8-9, 11-15, 21, and 26-30 stand pending. Claims 1, 8-9, 11-12, 14-15, 21, 26-27, and 30 stand rejected. Claims 12 and 15 stand objected to for informalities. Claims 13, 28, and 29 stand objected to as being dependent upon a rejected base claim. The Office admits that claims 13, 28, and 29 are otherwise allowable if rewritten in independent form.

Applicants amend claim 1 to more precisely recite the claimed compound. Support for the amendment can be found at least, for example, on page 30, lines 20-28, and page 31, lines 1-7; page 32, lines 9-28, full page 33, and page 34, lines 1-2; and full page 40 of the Specification. Furthermore, Applicants amend claims 12 and 15 to correct alleged informalities.

The claims have been amended without prejudice to, or disclaimer of, the cancelled subject matter. Applicants do not believe that the amendments add subject matter that is unsupported in the Specification as filed. Accordingly, no prohibited new matter is introduced by the entry of the amendments. Applicants reserve the right to file a continuation or divisional application on any subject matter canceled by way of amendments.

2. Status of the Rejection

Applicants note that the rejections stated in the Office Action mailed August 28, 2007 under 35 U.S.C. §§ 102(b) and 103(a) stand withdrawn. The finality of the prior Office Action was also withdrawn, because the Office asserts new grounds of rejection.

3. Acknowledgement of Information Disclosure Statement

Applicants note that the Office has not acknowledged the Information Disclosure Statement filed April 19, 2006. Applicants respectfully request the Office's acknowledgement with its next communication.

4. Claim Objections

The Office objects to claim 12 as allegedly containing the following informality: the claim is an independent claim which starts with "The method," and should begin with "A method." Applicants appreciate the Office's suggestion and amend claim 12 accordingly. Withdrawal of the objection is respectfully requested.

The Office objects to claim 15 as allegedly containing the following informality: the claim is drawn to a composition "comprising the 2-O-(β-D-Glucopyranosyl) ascorbic acid." Applicants appreciate the Office's suggestion and amend claim 12 accordingly. Withdrawal of the objection is respectfully requested.

5. Rejection of the Claims Under 35 U.S.C. § 101

The Office rejects claim 1 under 35 U.S.C. § 101 as allegedly directed to non-statutory subject matter. Specifically, the Office alleges that claim 1 may read on a product of nature, such as the compound present in *Lycium genuse* extracts.

Applicants note that compounds regularly are claimed and patented without such descriptions. However, without acquiescing as to the merits of the Office's rejection, Applicants have amended the claim to recite "isolated and/or purified" as indicated by the Examiner. Accordingly, the rejection is mooted and should be withdrawn

6. Rejection of the Claims Under 35 U.S.C. § 102

The Office rejects claims 1 and 8-9 under 35 U.S.C. § 102(b) as allegedly anticipated by Japanese Patent Application No. 53-098954 ["JP 53-098954"] to Ishido et al. Specifically, the Office alleges that JP 53-098954 discloses (1) the compound 2-O-(β-D-glucopyranosyl)-L-ascorbic acid, (2) the use of acetyl groups to protect the sugar hydroxyl groups, (3) the method obtaining the 2-position L-ascorbic acid derivative by alkyl-etherifying the 3-hydroxyl group before reacting with the glucopyranose, and (4) that the compound has a biological activity like vitamin C. Applicants respectfully traverse. For the Office's convenience, Applicant attaches to this response an English translation of JP 53-098954 as **EXHIBIT 1**, which was cited in the Office Action dated February 13, 2008.

A prior art reference does not anticipate a claim unless the prior art discloses, explicitly or inherently, each and every element of the claim. In addition, such disclosure

must be sufficient to have placed a skilled artisan in the field in possession of it. *In re Spada*, 911 F.2d 705, 708, 15 U.S.P.Q.2d 1655, 1657 (Fed. Cir. 1990). In the same vein, such disclosure must be enabling. If the disclosure by a prior art reference fails to enable one of skilled in the art to reduce the disclosed invention to practice, such a non-enabled disclosure cannot be anticipatory. *In re Borst*, 345 F.2d 851, 855, 145 U.S.P.Q. 554, 557 (C.C.P.A. 1962); *accord Amgen Inc. v. Hoechst Marion Roussel Inc.*, 314 F.3d 1313, 1354, 65 U.S.P.Q.2d 1385, 1416 (Fed. Cir. 2003). A non-enabled disclosure cannot suffice as § 102 prior art. *See In re Donohue*, 766 F.2d 531, 226 U.S.P.Q. 619 (Fed. Cir. 1985).

Applicants traverse the rejection. Applicants submit and present evidence in the form of a Declaration pursuant to 37 C.F.R. § 1.132 to show demonstrate that JP 53-098954 lacks an enabling disclosure regarding how to manufacture β-D-glucopyranosyl)-L-ascorbic acid.

As discussed in the attached declaration of Dr. Fukami, the materials and methods for making the compound, as disclosed in JP 53-098954, would fail to produce the 2-O-substituted product claimed in the instant application. *See* Declaration, ¶¶ 11-15. Dr. Fukami concludes that while the JP 53-098954 reference describes 2-O-(β-D-glucopyranosyl)ascorbic acid along with various ascorbic acid derivatives, it provides no teaching or concrete examples that actually are capable of synthesizing 2-O-(β-D-glucopyranosyl)ascorbic acid. It is believed that even with actual synthesis by the process described for those examples in JP 53-098954, the products yielded would be a 3-hydroxyl group that would be preferentially glucosylated, which would subsequently produce 2,3-diglucosides with glucosylation at the 2-position with respect to the 3-glucosylated product. With these types of products, it would have been impossible to obtain a product with β-glucosylation only at the 2-position using the described procedures in JP 53-098954. Declaration, ¶¶ 11-15.

The Office asserts that "on page 10 of Ishido et al. they discuss protecting the 3-position hydroxyl to obtain a compound which only has the sugar bonded to the 2-position of the ascorbic acid, thus producing the instantly claimed compound." Office Action, pages 3-4. Respectfully, the Office has misconstrued or misunderstood the teachings of JP 53-098954. JP 53-098954 states that in order to obtain a product of glucopyranose bonded to the 2-position of ascorbic acid, L-ascorbic acid is previously reacted with a diazoalkane or the like at low temperatures to alkyl-etherify the 3-hydroxyl group, and thereafter may be reacted with the 1-carbonic ester derivative of glucopyranose. *See* first full para. on page 10 of the English language translation of JP 53-098954. However, to obtain the presently claimed

compound that has an unprotected 3-hydroxyl group of the ascorbic acid moiety, the ether linkage at position-3 must be cleaved without the glucoside bond at position-2 being destroyed. The glucoside bond is formed by an acetal linkage, which is labile under acidic conditions. Cleavage of the ether linkage at position 3 without destroying the acetal linkage at position-2 would be difficult, because the conditions required to cleave an ether linkage are harsher than those required to cleave an acetal linkage. Following this logic, it would have been apparent to the skilled artisan that it is not possible to produce the presently claimed compound using the procedures taught by JP 53-098954. In fact, the process described by JP 53-098954 cannot produce an unprotected compound that is also 2-O-substituted. There is no description in JP 53-098954 of having synthesized such a compound. In fact, all the examples of compounds produced in Examples 1-4 have 5,6-O-isopropyridene on the ascorbic acid residue, and further 2,3,4,6-tetra-O acetyl on the glucopyranosyl residue. JP 53-098954 does not teach a process by which all these protecting groups can be removed without destroying the glycoside bond on the ascorbic acid. Ishido only alleges that "it is necessary to eliminate the protecting groups under basic, neutral or weakly acidic conditions so as not to cleave the glycoside bond of ascorbic acid." *See* paragraph bridging pages 6-7.

Regarding claims 8-10, the Office asserts that JP 53-098954 also teaches to protect the sugar hydroxyl groups with acetyl groups." Office Action, page 4, paragraph 4. However, the compounds recited in claims 8-10 are novel intermediates for producing 2-O-(β-D-glucopyranosyl)ascorbic acid. As discussed in the attached Declaration by Dr. Fukami, JP 53-098954 fails to teach how to make 2-O-(β-D-glucopyranosyl)ascorbic acid, and therefore could not have taught how to make the presently claimed intermediates. *See* Declaration, ¶ 12.

For the above stated reasons, JP 53-098954 cannot serve as a reference under 35 U.S.C. § 102. For a reference to be anticipatory, it must be enabled. JP 53-098954 is not enabled in order to make and use the 2-O-(β-D-glucopyranosyl)ascorbic acid or its intermediates. Accordingly, the reference cannot be used to adduce a *prima facie* case of anticipation. Applicants respectfully request the withdrawal of the rejection in view of the above arguments and attached Declaration under § 1.132.

7. **Rejection of the Claims Under 35 U.S.C. § 103**

The Office rejects claims 1, 8-9, 11-12, 14-15, 21, 26-27, and 30 under 35 U.S.C. § 103(a) as allegedly obvious over JP 53-098954 as applied to claims 1, 8, and 9, and further in view of Sakai et al. (U.S. Patent No. 5,407,812) ["Sakai"]. Applicants respectfully traverse.

A finding of obviousness under 35 U.S.C. § 103 requires a determination of the scope and content of the prior art, the difference between the invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966).

Applicants traverse the rejection. The defects of Ishido are discussed *supra*. Applicants also want to additionally note that the 2-O-(β-D-glucopyranosyl)ascorbic acid has an increased stability, an extended half-life and a long lasting activity in the body compared to its corresponding α-isomer. These unexpected advantageous properties are discussed in Examples 8-13 of the specification. These properties were not discovered until the β-form was isolated from the *Lycium genuse* and characterized to have the unexpected properties.

JP 53-098954 when combined with Sakai also fails to teach or suggest the claims. Sakai fails to cure the defects of JP 53-098954 on how to obtain 2-O-(β-D-glucopyranosyl)ascorbic acid, or its novel intermediates. In fact, Sakai specifically ***teaches away*** from making and/or using the β-form of ascorbic acid. The Office, however, ignores or misapplies the alleged prior art. Once again, Applicants respectfully direct the Office's attention to the relevant part of Sakai:

> **Studies on the *β*-D-glucopyranosyl type derivatives of L-ascorbic acid confirmed that they hardly exhibit desired physiological activities in living body, especially, in humans.** Furthermore, conventional organic chemical process have the drawbacks that they are inferior in economical efficiency because the reaction is very complicated and low in yield, and the establishment of non-toxicity and safeness for the resultant derivatives is very difficult.

See col. 2, lines 46-54 of Sakai (emphasis added). Sakai does not teach or suggest any β-transferase which is capable of acting on a β-form counterpart of the starting compound under the same conditions under which the α-transferase of Sakai acts on the α-form starting material. There is no reason for Sakai to teach that information, because it teaches away from

β –forms. Also, enzymes that act on α-forms of a specific compound cannot always act on the β-isoform of the same compound in the same manner. For all these reasons, Sakai alone or combined with JP 53-098954 cannot teach the claims.

In fact, JP 53-098954 does not substantially describe the β-form compound, let alone the various advantageous properties of the compound as a vitamin C derivative. These properties are not known or alluded to by Sakai either. Again, there would have been no apparent motivation to make and use this compound, let alone an expectation of success that such a compound would have the unexpected properties characterized by Applicants. As discussed *supra*, Sakai specifically teaches away from the β-form. Thus, the references stand alone and would not have been combined by a skilled artisan to teach what is alleged by the Office, when the references are viewed for their teachings as a whole.

Furthermore, Applicants have ***also previously*** directed the Office's attention to the prior art reference by Norio Muto et al., *Formation Of A Stable Ascorbic Acid 2-Glucoside By Specific Transglucosylation With Rice Seed A-Glucosidase*, 54 AGRIC. BOIL. CHEM. 1697 (1990) ["Muto"]. Muto is the only identified report of an enzymatic synthesis of a β-form D-glucopyranosyl L-ascorbic acid derivative. However it was the production of a 6-O-(β-D-glucopyranosyl)ascorbic acid. Muto also ***teaches away*** from enzymatic synthesis of the claimed β-compound. The Office, however, ignores Muto along with Sakai. Once again, Applicants respectfully direct the Office's attention to the relevant parts of Muto. On page 1700 of Muto, Table II presents properties of glucosides formed using several types of glycosidases, including both α- and β-specific transferases.

Table II. SPECTRAL AND REDUCING PROPERTIES OF GLUCOSIDES FORMED WITH GLYCOSIDASES

UV absorption spectra were measured at pH 2.0 and 7.0. Reducibility was measured by using cytochrome *c* and DCIP as electron acceptors. Samples were assayed before and after acid hydrolysis in 1 N HCl for 2 min at 100°C.

Glucoside (Enzyme used)	UV_{max}		Reducibility	
	pH 2.0	pH 7.0	Intact	Treated
AA	243	265	+	+
AA-6G (standard)	243	265	+	+
AA-2G (standard)	238	260	–	+
Glucosylated AA				
(Rat α-glucosidase)	238	260	–	+
(Rice α-glucosidase)	238	260	–	+
(Almond β-glucosidase)	243	265	+	+

The glucoside formed using β-specific glucosidase exhibits properties identical to those of 6-O derivative (emphasis added). Muto explicitly discusses the identity of such glucoside on the same page:

> **On the other hand, the glucoside formed with β-glucosidase had the same UV absorption profile and reducibility as AA-6G, indicating that the glucose is bound to the 5- or 6-position of AA in the β-configuration.**

The present application also provides a summary of Muto on page 5, lines 7-17 of the Specification. The skilled artisan, when combining Muto with Sakai, only would have been led further away from the subject matter of the present claims.

Applicants hereby traverse the Office's rejection by categorizing the claims into three following groups.

Claims 1 and 8-9

The Office's rejection of claims 1 and 8-9 entirely rests on JP 53-098954. The Office alleges that (1) claim 1 is drawn to a 2-O-(β-D-glucopyranosyl)-L-ascorbic acid compound, (2) claims 8-9 provide the saccharide with acetyl groups in the 2', 3', 4', and 6'-positions of the sugar, (3) JP 53-098954 discloses the compound 2-O-(β-D-glucopyranosyl)-L-ascorbic acid, and (4) JP 53-098954 discloses the protection of sugar hydroxyl groups with acetyl groups.

To establish a *prima facie* case of obviousness, both the suggestion of the claimed invention and the expectation of success must be in the prior art. *In re Dow Chem. Co.*, 837 F.2d 469, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988). Based on the teaching away by Sakai that β-D-glucopyranosyl type derivatives of ascorbic acid lack desirable properties in humans, an ordinary skilled artisan would have been discouraged from making and/or using such derivatives. Furthermore, there is no expectation of success based on JP 53-098954.

The Japanese patent also lacks an enabling disclosure. As discussed in the attached declaration of Dr. Fukami, JP 53-098954 fails to teach synthesis of 2-O-(β-D-glucopyranosyl)-L-ascorbic acid. Declaration, ¶¶ 11-15. In addition, it would have been apparent to a skilled artisan that it is impossible to synthesize ascorbic acid derivatives with an unprotected 3-hydroxyl group based on the method described by JP 53-098954 as disclosed above. Therefore, a person of ordinary skill in the art would not have been

successful in making the presently claimed compounds based on the alleged disclosure in JP 53-098954. Accordingly, the Office's rejection of claims 1 and 8-9 is improper.

Claims 14-15, 21, 26, and 30

The Office alleges claims 14-15, 21, 26-27, and 30 are drawn to various forms of compositions comprising the active agent. Applicants point out once again that the Office erroneously characterizes claim 27 as a composition claim. Claim 27 is actually a method claim dependent on claim 11.

The Office alleges that (1) Sakai teaches to use various compositions comprising α-D-glucopyranosyl type derivatives of L-ascorbic acid, and (2) JP 53-098954 teaches the compound as vitamin C derivatives. The Office admits that JP 53-098954 does not teach compositions comprising 2-O-(β-D-glucopyranosyl)-L-ascorbic acid.

Applicants traverse the rejection. Applying the framework of *Graham*, the Office's rejection of alleged composition claims 14-15, 21, 26, and 30 amounts improper hindsight. For an obviousness analysis, inherency is immaterial if the record establishes that one of ordinary skill in the art would not appreciate or recognize the inherent feature. *In re Shetty*, 566 F.2d 81, 195 U.S.P.Q. 753 (C.C.P.A. 1977). An ordinarily skilled artisan would not have appreciated or recognized the superior properties of the compound 2-O-(β-D-glucopyranosyl)-L-ascorbic acid, because (1) it is only in the present application that such properties have been first discovered, and (2) Sakai teaches that β-D-glucopyranosyl type derivatives of ascorbic acid lack desirable properties in human. A person of ordinary skill in the art is merely one of "ordinary creativity" and "not an automaton." *KSR Int'l Co. v. Teleflex, Inc.*, 82 U.S.P.Q.2d 1385, 1397 (2007). A person of ordinary skill in the art would *not* expect the superior properties of 2-O-(β-D-glucopyranosyl)-L-ascorbic acid, and hence would not have to make or use such compositions. Nothing in any of the alleged references suggest how to make the compounds or their unexpected properties. Accordingly, the Office has not advanced a *prima facie* case of obviousness for claims 14-15, 21, 26, and 30.

Furthermore, the Office relies on *Ex parte Erdmann*, 194 U.S.P.Q. 96 (Bd. Pat. App. & Int. 1975) and *Ex parte Douros*, 163 U.S.P.Q. 667 (Bd. Pat. App. & Int. 1968) to reject the composition claims. For the sake of the record, Applicants point out that the Office has misapplied both cases. *Erdmann* holds that the new use of old composition does not render the composition patentable. The Office explicitly admits that that JP 53-098954 does not

teach compositions comprising 2-O-(β-D-glucopyranosyl)-L-ascorbic acid. Page 4, Office Action. *Erdmann*, therefore, does not apply to the facts of the present application. *Douros* holds that addition of a carrier to an unpatentable compound does not render the composition patentable. Applicants have provided sufficient evidence that the presently claimed compounds are patentable. Accordingly, *Douros* is irrelevant.

Claims 11-12 and 27

The Office alleges that claims 11 and 12 are drawn to methods of making the product using a glucosyltransferase. Applicants group claim 11-12 and 27 together, because, as mentioned above, claim 27 actually depends on the method claim 11. The Office alleges that it would be obvious to make the instantly claimed β-form with a β-specific glucosyltransferase. The Office's allegation rests on that (1) Sakai teaches methods of making α-D-glucopyranosyl type derivatives of L-ascorbic acid using α-transferase, and (2) the presence of both 2-O and 6-O derivatives using β-specific glucosyltransferase is inherent within the method taught. The Office admits that JP 53-098954 does not teach to make the L-ascorbic acid derivatives with a saccharide transferring enzyme.

Applicants traverse the rejection of claims 11-12 and 27. Applying the framework of *Graham*, the Office's rejection of claims 11-12 and 27 amounts improper hindsight. In an obviousness analysis where the scope and content of the prior art have been determined, the relevant inquiry is whether the prior art suggests the invention, and whether one of the ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). Neither Sakai nor JP 53-098954 suggests the synthesis of β-D-glucopyranosyl type derivatives using a β-specific enzyme. Sakai, on the contrary, explicitly teaches away the synthesis of β-D-glucopyranosyl type derivatives. Muto further discloses the failure to synthesize β-D-glucopyranosyl type derivatives with a β-specific enzyme. A person of ordinary skill in the art, in view of the teaching of Muto, would not have had a reasonable expectation of success. Again, a person of ordinary skill in the art is merely one of "ordinary creativity" and "not an automaton." *KSR*, at 1397. Thus, one ordinarily skilled would *not* regard it obvious to successfully obtain 2-O-(β-D-glucopyranosyl)-L-ascorbic acid with a β-specific saccharide transferring enzyme. Moreover, the Office's allegation that an obvious method renders its products obvious does not apply to the present application. The present

application is the first to use a β-specific enzyme to successfully synthesize β-D-glucopyranosyl type derivatives. Such successes are unexpected in view of Muto, so that the method is not obvious.

Applicants here discovered in a plant of *Lycium genuse* the compound 2-O-(β-D-glucopyranosyl)ascorbic acid. To develop the enzymatic synthesis method, as recited in claims 11-12, it was required to find a specific β-transferase as set forth in Example 5. Then the specific conditions for carrying out the enzymatic reaction needed to be determined. This is exemplified in Example 6 of the specification, which sets out the pH and calcium requirements. Again, the details were not taught or suggested by any of the references relied upon by the Office.

Accordingly, the Office improperly rejects claims 11-12 and 27 as unpatentable for obviousness.

Accordingly, the Office's no *prima facie* case of obviousness has been addressed with regard to claims 1, 8-9, 11-12, 14-15, 21, 26-27, and 30. Thus, Applicants respectfully request withdrawal of the rejection, and allowance of the claims.

CONCLUSION

In view of the above arguments and amendments to the claims, Applicants respectfully assert that the claims are condition for allowance and respectfully request a Notice of Allowance.

Should any issues remain outstanding or if there are any questions concerning this paper, or the application in general, the Examiner is invited to telephone the undersigned representative at the Examiner's earliest convenience. Should any outstanding fees be owed or overpayments credited, the Commissioner is invited to charge or credit Deposit Account No. 50-0573 accordingly.

Respectfully submitted,

Date: May 12, 2008

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EXHIBIT 1

(translation)

SPECOFICATION

1. Title of the Invention

L-Ascorbic acid derivatives

2. Claims

(1) An L-ascorbic acid derivative represented by the general formula (I):

(I)

[wherein R is a hydrogen atom or a protecting group for a hydroxyl group; and R_1 is a hydrogen atom, a lower alkyl group, a benzyl group or a residue represented by the formula (II):

(II)

(wherein R is the same as defined above and may be different from one another), and at least one of R_1 is a residue represented by the formula (II)].

(2) The L-ascorbic acid derivative of claim 1, wherein one of the two Rs in the general formula (I) is an alkylene group.

3. Detailed Explanation of the Invention

The present invention relates to L-ascorbic acid derivatives, and specifically it relates to derivatives obtained by bonding at least one glucose to L-ascorbic acid.

It is well known that L-ascorbic acid is an essential

nutrient as vitamin C to humans. However, it is also well known that L-ascorbic acid is labile for oxydation and easily loses its effect when used in drugs and the like. On account of this, many studies on the stabilization of L-ascorbic acid have been conducted but no satisfactory method has yet been found.

As a result of rigorous investigations in consideration of the above described circumstances by the present inventors, a novel L-ascorbic acid derivatives has been found and the present invention has been attained.

Specifically, the gist of the present invention resides in L-ascorbic acid derivatives represented by the general formula (I):


(I)

[wherein R is a hydrogen atom or a protecting group for a hydroxyl group; and R_1 is a hydrogen atom, a lower alkyl group, a benzyl group or a residue represented by the formula (II):


(II)

(wherein R is the same as defined above and may be different from one another), and at least one of R_1 is a residue represented by the formula (II)].

The present invention will now be explained in detail.

In the above described general formula (I), R is a hydrogen atom or a protecting group for a hydroxyl group. The protecting group for a hydroxyl group is a well-known group including, for example, an acyl group such as acetyl, trifluoroacetyl, trichloroacetyl, benzoyl and p-nitrobenzoyl and a hydrocarbon group such as methyl and benzyl. Further, one of the two Rs may be an alkylene group such as 1,1-ethylene, 2,2-propylene

and 2,2-butylene.

In the point that the compounds of the present invention can achieve the effect of vitamin C, R is preferably a hydrogen atom.

Further, R_1 is a hydrogen atom, a lower alkyl group , a benzyl group or a residue represented by the formula (II),

(II)

(wherein R is the same as defined above). The lower alkyl group includes, for example, methyl and ethyl. The residue represented by the formula (II) includes, for example, D-glucopyranosyl, 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl, 2,3,4,6-tetra-O-benzoyl-D-glucopyranosyl, 2-benzoyl-3,4,6-tri-O-acetyl-D-glucopyranosyl, 2,3,4,6-tetra-O-methyl-D-glucopyranosyl, 2,3,4,6-tetra-O-benzyl-D-glucopyranosyl and 2,3;4,6-di-O-ethylidene-D-glucopyranosyl. The residue represented by the formula (II) is normally bonded in β-linkage.

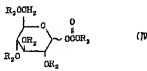
The ascorbic acid derivatives represented by the general formula (I) include, for example, 2,3-di-O-(β-D-glucopyranosyl)-L-ascorbic acid, 2-O-(β-D-glucopyranosyl)-L-ascorbic acid, 3-O-(β-D-glucopyranosyl)-L-ascorbic acid, 2,3-di-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-ascorbic acid, 3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-2,3-di-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-2-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-3-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-2,3-di-O-(2,3,4,6-tetra-O-actyl-β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-3-O-(2,3,4,6-tetra-O-actyl-β-D-glucopyranosyl)-L-ascorbic acid, 2-O-methyl-3-O-(β-D-glucopyranosyl)-L-ascorbic acid,

3-O-methyl-2-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-2-O-methyl-3-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isopropylidene-2-O-methyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-ascorbic acid, 2-O-benzyl-3-O-(β-D-glucopyranosyl)-L-ascorbic acid, 3-O-benzyl-2-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isoprpylidene-2-O-benzyl-3-O-(β-D-glucopyranosyl)-L-ascorbic acid, 5,6-O-isoprpylidene-2-O-benzyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-ascorbic acid, 5,6-di-O-acetyl-2,3-di-O-(2,3,4,6-tetra-O-acetyl-β-D-gluco-pyranosyl)-L-ascorbic acid, 5,6-di-O-benzoyl-2,3-di-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-L-ascorbic acid and 5,6-O-isoprpylidene-2,3-di-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-L-ascorbic acid

The L-ascorbic acid derivatives of the present invention can be produced, for example, by the following method. More specifically, the L-ascorbic acid derivatives can be obtained by reacting L-ascorbic acid whose 5- and 6-hydroxyl groups are protected represented by the general formula (III):

(III)

(wherein R_2 is a protecting group for a hydroxyl group) with the 1-carbonic ester derivative of D-glucopyranose represented by the general formula (IV):



(IV)

(wherein R_2 is the same as defined above and R_3 is a hydrocarbon group or a halogenated hydrocarbon group). By etherifying the 2- or 3-hydroxyl group of this reaction product with a diazoalkane, a dialkylsulfuric acid or the like and, if necessary or required, eliminating the protecting groups for hydroxyl groups by the well known method, the L-ascorbic acid

derivatives represented by the general formula (I) can be obtained.

As the R_2 in the general formulae (III) and (IV), the same groups as the above described protecting groups for hydroxyl groups can be mentioned. R_3 includes, for example, an alkyl group having 1 to about 10 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl and tert-butyl, an aryl group such as phenyl and a halogenated alkyl group such as trifluoroethyl and trichloroethyl.

The L-ascorbic acid whose 5,6-hydroxyl groups are protected represented by the general formula (III) is produced, for example, by reacting L-ascorbic acid with acetone saturated with hydrogen chloride at room temperature [see Experientia, 19, 619 (1963)].

The 1-carbonic ester derivative of glucopyranose represented by the general formula (IV) can be produced, for example, by reacting a sugar such as 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose whose hydroxyl groups other than the 1-hydroxyl group are protected with the corresponding halogenated carbonic ester such phenyl chlorocarbonate in the presence of a base such as pyridine.

The 1-carbonic ester derivative of glucopyranose has an α-form and a β-form, and both can be used.

The method of reacting the L-ascorbic acid whose 5,6-hydroxyl groups are protected with the 1-carbonic ester derivative of glucopyranose is not particularly limited. Their molar ratio in reacting both is also not particularly limited, and when both are reacted in nearly equal amounts, the reaction product mainly comes to a 1:1 reaction product, and when the 1-carbonic ester derivative of glucopyranose is used in an amount of at least two times the equivalent amount, a 1:2 reaction product is mainly obtained. In this instance, the 1:1

reaction product mainly comes to a product of glucopyrasnose bonded to the 3-position of L-ascorbic acid.

In order to obtain a product of glucopyranose bonded to the 2-position of ascorbic acid, L-ascorbic acid is previously reacted with a diazoalkane or the like at low temperatures to alkyl-etherify the 3-hydroxyl group and thereafter may be reacted with the 1-carbonic ester derivative of glucopyranose.

The use of an aprotic solvent inert to the reaction such as chlorobenzene, acetonitilile and nitrobenzene gives a preferable result.

The reaction temperature is normally 60 to 150°C, preferably 80 to 130°C.

The reaction time varies depending on the reaction temperature used and the type of the reaction material selected, and is typically about two to six hours.

In the reaction, the generation of carbondioxide is seen in this reaction, and thus the reaction may be carried out until the generation of the gas is completed or until a theoretical amount of the gas is generated.

The reaction system may be under normal pressures or can be under reduced pressure. Under reduced pressure, side reactions are favorably decreased. The reduced pressure may be about 100 mmHg, and is preferably about 20 to 30 mmHg.

In order to alkyl-etherifying the 2- or 3-hydroxyl group of this product, the product may be reacted with a diazoalkane such as diazomethoane or a dialkylsulfuric acid such as dimethylsulfric acid by the conventional method.

Further, the elimination of the protecting groups for hydroxyl groups may be performed with the respective protecting

groups by the well known method. However, it is necessary to eliminate the protecting groups under basic, neutral or weakly acidic conditions so as not to cleave the glycoside bond of ascorbic acid and conditions using a strong acid such as hydrochloric acid and sulfuric acid have to be avoided.

Then, the ascorbic acid derivatives thus produced can be purified and isolated by combining the well known purification methods including, for example, solvent distillation, recrystallization, filtering, column chromatography treatment and active carbon treatment.

The compounds relating to the present invention have a physiological activity as vitamin C and improved stability against oxidation, and accordingly can be used in drugs and the like.

Simultaneously, the compounds relating to the present invention has a natural substance as their constituting part, and thus their toxicity can hardly be recognized.

The present invention will now be explained in more detail by giving examples but the scope of the present invention is not limited by the examples.

Example 1

Nine hundred forty milligrams (2 mmol) of 2,3,4,6-tetra-O-acetyl-1-O-phenoxycarbonyl-β-D-glucopyranose prepared by the method described in the specification of Japanese Patent Application No. S51-19791 (JP-A S52-102219) and 475 mg (2.2 mmol) of 5,6-O- isopropylidene-L-ascorbic acid were melted and reacted under reduced pressure of an aspirator at 120 to 130°C for three hours. The reaction mixture was separated by silica gel chromatography (chloroform) to obtain 246 mg (28%) of 5,6-O-isopropylidene-2,3-di-O-(2,3,4,6-tetra-O-acetyl-β-D- glucopyranosyl)-L-ascorbic acid (hereinafter referred to as "A") and 625 mg (57%) of 5,6-O-isopropylidene-3-O-(2,3,4,6-

tetra-O-acetyl-β-D-glucopyranosyl)-L-ascorbic acid (hereinafter referred to as "B").

Properties of (A)

m.p.: 95 to 97°C (recrystallized from ethanol-ethyl ether)

$[\alpha]_D^{22}$ = -12° (C 1, chloroform)

Elemental Analysis Value

For $C_{37}H_{48}O_{26}$ Calculated: C 50.68%; H 5.51%

Found: C 50.68%; H 5.48%

^{13}C-NMR ($CDC\ell_3$, δ value, ppm): C-1 of glucose 98.4, 96.4

Properties of (B)

m.p.: 157 to 160°C (recrystallized from ethanol-ethyl ether)

$[\alpha]_D^{22}$ = +16° (C 0.5, chloroform)

Elemental analysis Value

For $C_{23}H_{30}O_{16}$ Calculated: C 50.54%, H 5.43%

Found: C 50.27%, H 5.48%

^{13}C-NMR ($CDC\ell_3$, δ value, ppm): C-1 of glucose 98.9; C-2, 70.9; C-3, 73.4; C-4, 68.1; C-5, 72.4; C-6, 61.6

Example 2

Five hundred seventy-nine milligrams (1.1 mmol) of 2,3,4,6-tetra-O-acetyl-1-O-(2,2,2-trichloroethoxycarbonyl-β-D-glucopyranose prepared by the method described in the specification of Japanese Patent Application No. S51-19791 and 109 mg (0.5 mmol) of 5,6-O-isopropylidene-L-ascorbic acid were melted and reacted under reduced pressure of an aspirator at 130 to 140°C for four hours. The reaction mixture was purified by the same method as in Example 1 to obtain (A) in a 62% yield.

Example 3

Two hundred sixty-three milligrams (0.5 mmol) of 2,3,4,6-tetra-O-acetyl-1-O-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranose and 133 mg (0.6 mmol) of 5,6-O-isopropylidene-L-ascorbic acid were dissolved in 4 ml of nitromethane and refluxed under heating at normal pressures for four hours. The

reaction product was concentrated under reduced pressure, and purified by the same method as in Example 1 to obtain 14 mg (6%) of (A) and 164 mg (60%) of (B).

Example 4

Two hundred sixty-two milligrams (0.5 mmol) of 2,3,4,6-tetra-O-acetyl-1-O-(2,2,2-trichloroethoxycarbonyl-β-D-glucopyranose and 147 mg (0.7 mmol) of 5,6-O-isopropylidene-L-ascorbic acid were dissolved in 2 ml of nitromethane and refluxed under heating at normal pressures for six hours. To the reaction product, excess diazomethane was added and the resulting mixture was left to stand at room temperature for one hour and purified by the same method as in Example 3 to obtain 203 mg of 5,6-O-isopropylidene-2-O-methyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-ascorbic acid.

Amorphous Form, $[\alpha]_D^{22} = -6°$ (C 1.7, chloroform)

Elemental Analysis Value

For $C_{24}H_{32}O_{16}$ Calculated: C 51.42%; H 5.75%

Found: C 51.23%, H 5.79%

^{13}C-NMR (CDCℓ_3, δ value, ppm): C-1 of glucose 98.7; C-2, 70.9
C-3, 73.3; C-4 68.3; C-5, 72.3
C-6, 61.8

^{1}H-NMR (CDCℓ_3, δ value, ppm): 3.93 (-C-C$\underline{H_3}$)